Applicants respectfully submit that the rejection of claims 1, 8, 10-36 and 42-72 under 35 U.S.C. 103(a) over Erlander et al (WO 95/13369) in view of New England Biolabs catalog (page 11) (1993/1994 catalog) is unwarranted and should be withdrawn.

Second Rejection Under 35 U.S.C. 103 (a): The Deficiencies Of The References Are Not Cured By Addition of Kato et al.

Claims 1-4, 8, 10-36 and 42-72 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Erlander et al (WO 95/13369) in view of New England Biolabs catalog (page 11) (1993/1994 catalog) and further in view of Kato et al. (EP 735 144 Al). As noted above, the combination of Erlander et al. and NEB does not teach or suggest the present invention. The addition of the Kato et al. reference does not cure this deficiency. Kato et al. teaches the ligation of biotinylated double stranded cDNA adapters to double stranded cDNA fragments produced by multiple digests with class IIS restriction enzymes. Applicants respectfully point out that the method of Erlander et al. lacks a step of ligation of double stranded cDNA adapters to double stranded cDNA fragments, and does not suggest the addition of such a step. Furthermore, the method of the present invention does not teach or claim the step of ligation of double stranded cDNA adapters to double stranded cDNA fragments. Thus, there would be no motivation for one of skill in the art to combine the teachings of the references to arrive at the present claimed invention.

Applicants respectfully submit that the rejection of claims 1-4, 8, 10-36 and 42-72 under 35 U.S.C. 103(a) over Erlander et al (WO 95/13369) in view of New England Biolabs catalog (page 11) (1993/1994 catalog) and further in view of Kato et al. is unwarranted and should be withdrawn.

Third Rejection Under 35 U.S.C. 103 (a): The Deficiencies Of The References Are Not Cured By Noronha et al.

Claims 1, 5-7, 8, 10-36 and 42-72 are rejected in the Office Action mailed October 18, 2000 under 35 U.S.C. 103(a) as being unpatentable over Erlander et al (WO 95/13369) in view of New England Biolabs catalog (page 11) (I 993/1994 catalog) and further in view of Noronha et al (PCR Methods Appl (1992) 2:131-136). As noted above, the combination of Erlander et al. and NEB does not teach or suggest the present invention. The addition of the Noronha et al. reference does not cure this deficiency.

Applicants respectfully submit that the rejection of claims 1, 5-7, 8, 10-36 and 42-72 under 35 U.S.C. 103(a) over Erlander et al (WO 95/13369) in view of New England Biolabs catalog (page 11) (1993/1994 catalog) and further in view of Noronha et al. is unwarranted and should be withdrawn.

Fourth Rejection Under 35 U.S.C. 103 (a): The Deficiencies Of The References Are Not Cured By Ju et al.

Claims 1, 8-36 and 42-72 are rejected in the Office Action mailed October 18, 2000 under 35 U.S.C. 103(a) as being unpatentable over Erlander et al (WO 95/13369) in view of New England Biolabs catalog (page 11) (1993/1994 catalog) and further in view of Ju et al (Anal. Biochem. (1995) 231:131-140). As noted above, the combination of Erlander et al. and NEB does not teach or suggest the present invention. The addition of the Ju et al. reference does not cure this deficiency.

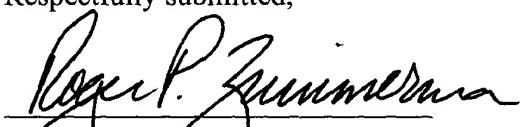
Applicants respectfully submit that the rejection of claims 1, 1, 8-36 and 42-72 under 35 U.S.C. 103(a) over Erlander et al (WO 95/13369) in view of New England Biolabs catalog (page 11) (1993/1994 catalog) and further in view of Noronha et al. is unwarranted and should be withdrawn.

CONCLUSION

For the foregoing reasons, the Applicants respectfully assert that the claims are in proper condition for allowance, and request that the Examiner grant the application.

Please contact Roger P. Zimmerman at (312) 913-2101, if there are any questions regarding this matter. Please debit or credit any fees occasioned by this paper to Deposit Account No. 13-2490.

Respectfully submitted,

Date: April 24, 2001 By: 

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Not I

RR NEB3 BSA 37° Yes

NEBuffer	1	2	3	4
% Activity	0	75	**100**	50

#189S 500 units **$55**
#189L 2,500 units **$220**
order #189CL for high (5X) concentration

5′...GC▼GGCCGC...3′
3′...CGCCGG▲CG...5′

Source: An *E. coli* strain that carries the cloned *Not* I gene from *Nocardia otitidis-caviarum*

Reaction Buffer: NEBuffer 3 + BSA
100 mM NaCl, 50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol (pH 7.9 @ 25°C). Supplement with 100 µg/ml BSA. Incubate at 37°C.

Ligation and Recutting: After 25-fold overdigestion with *Not* I, > 95% of the DNA fragments can be ligated and recut.

Concentration: 10,000 and 50,000 units/ml.
Assayed on Adenovirus-2 DNA.

Storage Conditions: 200 mM NaCl, 20 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 0.15% Triton X-100, 200 µg/ml BSA, and 50% glycerol. Store at –20°C.

Diluent Compatibility: Diluent C, see page 66.

Heat Inactivation: 65°C for 20 minutes.

Note: Supercoiled plasmids may require up to 5-fold more *Not* I for complete digestion than linear DNAs.

Nru I

NEB4 37° dam Yes

NEBuffer	1	2	3	4
% Activity	0	10	75	10

#192S 1,000 units **$44**
#192L 5,000 units **$176**
order #192CS or CL for high (5X) concentration

5′...TCG▼CGA...3′
3′...AGC▲GCT...5′

Source: *Nocardia rubra* (ATCC 15906)

Reaction Buffer: NEBuffer *Nru* I
100 mM KCl, 50 mM Tris-HCl, 10 mM $MgCl_2$, (pH 7.7 @ 25°C). Incubate at 37°C.

Ligation and Recutting: After 20-fold overdigestion with *Nru* I, less than 20% of the DNA fragments can be ligated.

Concentration: 10,000 and 50,000 units/ml.
Assayed on λ DNA.

Storage Conditions: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA, and 50% glycerol. Store at –20°C.

Diluent Compatibility: Diluent A, see page 66.

Heat Inactivation: 65°C for 20 minutes.

Note: Blocked by overlapping *dam* methylation (see page 269).

Nsi I

RR NEB4 37° Yes

NEBuffer	1	2	3	4
% Activity	25	25	50	10

#127S 1,000 units **$50**
#127L 5,000 units **$200**

5′...ATGCA▼T...3′
3′...T▲ACGTA...5′

Source: An *E. coli* strain that carries the cloned *Nsi* I gene from *Neisseria sicca* (NEB 913)

Reaction Buffer: NEBuffer *Nsi* I
100 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol (pH 8.4 @ 25°C). Incubate at 37°C.

Ligation and Recutting: After 50-fold overdigestion with *Nsi* I, > 95% of the DNA fragments can be ligated and recut.

Concentration: 10,000 units/ml.
Assayed on λ DNA.

Storage Conditions: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA, and 50% glycerol. Store at –20°C.

Diluent Compatibility: Diluent A, see page 66.

Heat Inactivation: 65°C for 20 minutes.

Note: *Nsi* I is an isoschizomer of *Ava* III.

Nsp I

RR NEB2 BSA 37° Yes

NEBuffer	1	2	3	4
% Activity	100	**100**	0	100

#602S 250 units **$50**
#602L 1,250 units **$200**

5′...Pu C A T G▼Py...3′
3′...Py▲G T A C Pu...5′

Source: An *E. coli* strain that carries the cloned *Nsp* I gene from *Nostoc* species C

Reaction Buffer: NEBuffer 2 + BSA
50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol (pH 7.9 @ 25°C). Supplement with 100 µg/ml BSA. Incubate at 37°C.

Ligation and Recutting: After 10-fold overdigestion with *Nsp* I, > 95% of the DNA fragments can be ligated and recut.

Concentration: 5,000 units/ml.
Assayed on λ DNA.

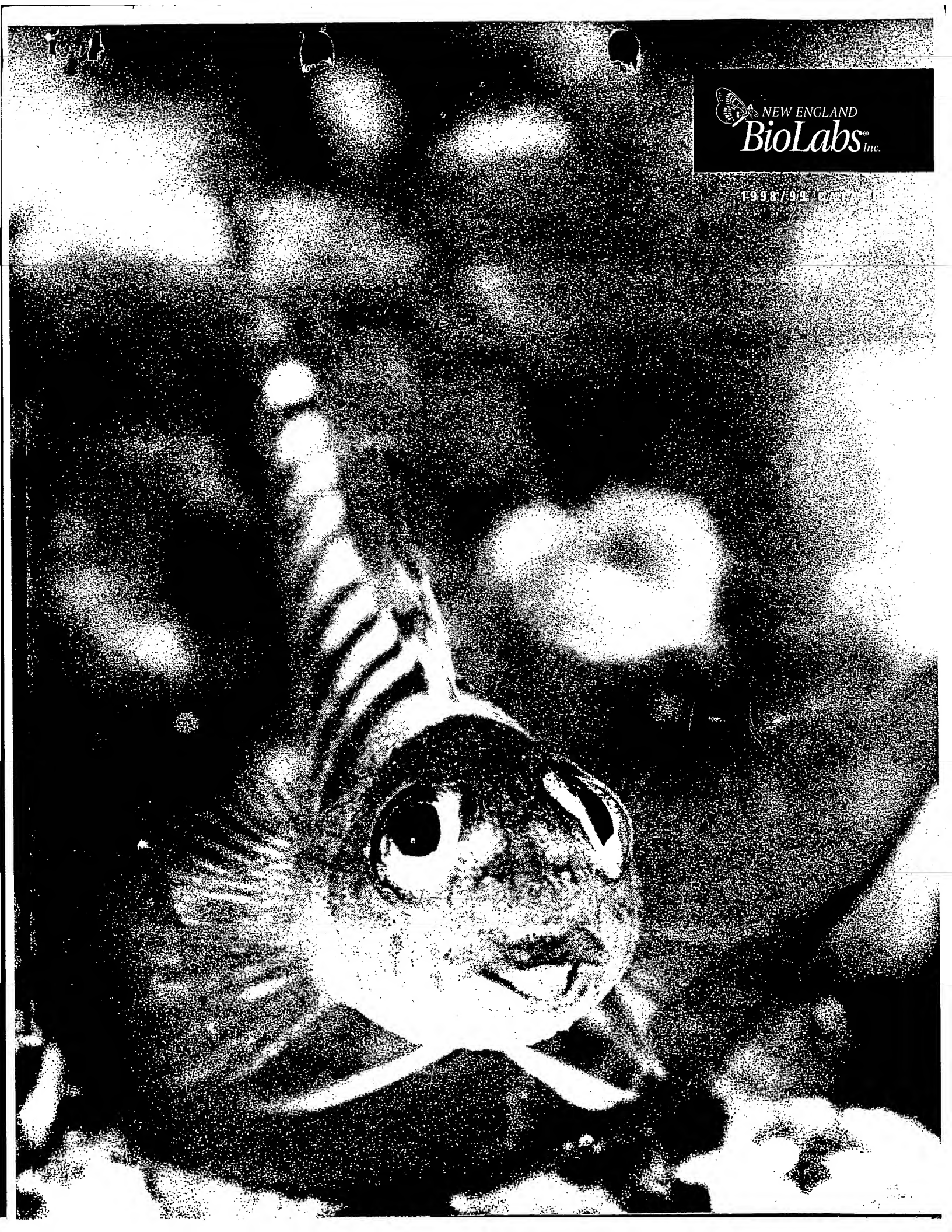
Storage Conditions: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 0.15% Triton X-100, 200 µg/ml BSA, and 50% glycerol. Store at –20°C.

Diluent Compatibility: Diluent A, see page 66.

Note: *Nsp* I dilutions must be supplemented with 0.15% Triton X-100.

Heat Inactivation: 65°C for 20 minutes.

Cloned at NEBiolabs | RR Recombinant Enzyme | NEB4 Optimum Buffer | 37° Incubation Temperature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